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Corticotropin-Releasing Factor Acts Via a Third Ventricle Site to Reduce Exploratory Behavior in Rats

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SPADARO, F., C. W. BERRIDGE, H. A. BALDWIN AND A. J. DUNN. *Corticotropin-releasing factor acts via a third ventricle site to reduce exploratory behavior in rats.* PHARMACOL BIOCHEM BEHAV **36**(2) 305–309, 1990.—Corticotropin-releasing factor (CRF, 20–25 ng) injected into the lateral or fourth ventricles of rats decreased exploratory behavior in the multicompartment testing chamber (MCC), as assessed by decreased mean contact times with novel stimuli. This result extends similar observations made previously in mice. To investigate the site of this action of CRF, cold cream plugs injected into the cerebral ventricles of rats were used to prevent access of the CRF to specific periventricular sites. When the cerebral aqueduct was blocked with cold cream, CRF injected into the lateral ventricle, but not the fourth ventricle, decreased exploratory behavior in the MCC. These results suggest that CRF does not act in the fourth ventricle to alter behavior in the MCC, and most likely acts in the lateral or third ventricles. Cold cream blocks within the third ventricle prevented the effect of lateral ventricle administration of CRF. The clearest effects were obtained when the anteroventral portion of the third ventricle (AV3V) had been coated with cold cream. This region, which contains the organum vasculosum laminae terminalis (OVLT), was the only region blocked that showed a significant statistical interaction between the cold cream block and the effect of CRF. This result suggests that the OVLT, or regions close to it, is the primary site of the behavioral action of CRF in the MCC. It is possible that the peptide could be taken up in this region and transported to another brain site.

Exploratory behavior Corticotropin-releasing factor (CRF) Cerebral aqueduct Third ventricle OVLT

CORTICOTROPIN-RELEASING factor (CRF), a 41-amino acid peptide, first discovered and characterized by Vale (21), is now recognized as the principal releasing factor for adrenocorticotropin from the pituitary (17). CRF appears to play a major role in the response of the organism to stress that is unrelated to its function as an activator of the hypothalamic-pituitary-adrenal axis [for review, see (8)]. Intracerebroventricular (ICV) administration of CRF elicits physiological and behavioral responses that resemble those observed in stress. The concentration of plasma catecholamines is increased because of stimulation of the sympathetic nervous system and the adrenal medulla, with a consequent increase in arterial pressure and heart rate (5). Firing of locus coeruleus neurons is increased (22), and there is an activation of cerebral catecholaminergic systems similar to that observed in stressful conditions (7,15). ICV CRF also exerts a wide variety of behavioral effects, it increases locomotor activity (19) and grooming (10, 16, 19); decreases feeding without altering drinking (16); and decreases sexual behavior (18). In a multicompartment chamber (MCC), ICV CRF reduced the mean time mice spent in contact with novel stimuli (3). This effect of ICV CRF resembles that observed following treatment of rats or mice with a variety of stressors including prior restraint (2,3). ICV administration of the CRF antagonist, alpha-helical CRF_{9-41} to mice dose-dependently reversed the effect of restraint in the MCC (3), strongly supporting the hypothesis that cerebral CRF is involved in the behavioral effects of stress in this behavioral paradigm.

An important next step in understanding the physiological role of CRF is to identify its site of action. Following a technique previously used (9, 11–13, 20), we have used injections of cold cream to block access of ICV administered CRF to parts of the ventricular system. We evaluated the capacity of the blocks to prevent the behavioral effect of CRF when administered into either the lateral or the fourth ventricles. We used rats for these studies because of their larger brain, facilitating the placement of cannulae.

METHOD

Male Sprague-Dawley rats (250–300 g) were bred in the University of Florida Animal Care Facility (experiments of Figs. 1

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and 2) or obtained from Harlan, Indianapolis (experiment of Fig. 4). They were group housed and maintained on a 12:12 light-dark cycle (lights on 7:00 a.m.). Food and water were available ad lib. Surgery was performed one week after their arrival as previously described (9). Animals were anesthetized with pentobarbital (60 mg/kg). Stainless steel guide cannulae 10 (lateral ventricle) or 16 mm long (other locations) were implanted stereotaxically in one of the lateral ventricles (coordinates L ±2.0, A-P −0.8, V −4.0 from dura), the third ventricle (L ±1.0, A-P −1.5, V −8.5), the cerebral aqueduct (L ±1.0, A-P −7.0, V −4.5 or L ±1.0, A-P −3.8, V −6.5 at 10° lateral), or in the fourth ventricle (L 0.0, A-P −10, V −6.0) for the injection of CRF or cold cream. The cannulae were secured to the skull with dental acrylic. Animals were allowed to recover for seven days before the injections were performed.

The behavioral apparatus was that designed by Arnsten and Segal (1). Briefly, it consisted of a chamber divided into nine interconnecting compartments. Each compartment contained a hole on the floor that contained a wire mesh sphere which served as a stimulus. The animal was placed in the chamber and observed for a 25-min period by a trained observer unaware of the specific location of the cannulae. Behaviors scored included measures of locomotor activity: the number of compartment entries and rears and the duration of the latter; the duration of periods of inactivity, the number and duration of contacts with the stimuli, and the incidence and duration of grooming. Scoring was enabled by an NEC 8201A lap-top computer modified for use as an event recorder by S&K Computer Products Ltd. (Buffalo, NY). Data were discarded if the cannulae were misplaced as indicated by the histological analysis, or if an individual rat was obviously in poor health, and/or displayed low locomotor activity (<20 rears or <100 compartment entries in the scoring period). CRF was a generous gift from Dr. Jean Rivier of the Salk Institute.

On the day before testing, animals were weighed and transported from the colony room and housed individually in the testing room for the remainder of the experiment. At about 5–6 p.m. on the day before the experiment, animals were injected with 4–8 μl of cold cream in the cerebral aqueduct or in the third ventricle. This waiting period was important because pilot experiments indicated that the stress associated with the injection procedure disrupted subsequent behavioral performance. For cold cream injection, a one ml syringe was filled with water and attached to a piece of a polyethylene tubing calibrated to hold 16 μl. The required volume of cold cream (Nivea) was drawn into the polyethylene tube via a short section of 23-gauge stainless steel tubing that was attached with a small piece of polyethylene tubing to the appropriate cannula in the skull. Ten minutes before the period of behavioral observation, the animal was injected with 20 ng of CRF dissolved in 2 μl of isotonic saline, or 2 μl of saline. The injection was performed using a 30-gauge cannula 10 mm long attached to a 10 μl syringe.

After the behavioral observation period, a small quantity of methylene blue dye was injected through the same cannula as the CRF. Five minutes later the animal was decapitated and the brain excised and fixed in 10% formalin. The formalin-fixed brain was frozen and sectioned sagittally on a freezing microtome to determine the presence, the location, and the extent of the block (complete or incomplete) of the cerebral aqueduct or the third ventricle. Photographs of such blocks were presented in a previous publication from this laboratory in which ACTH-induced grooming was studied (9).

The experiments of Figs. 1 and 2 were performed in Gainesville using rats bred in the University of Florida Animal Care facility, and the experiment of Fig. 4 was performed in Shreveport using rats of the same strain but supplied by Harlan. Statistical analyses were performed using analysis of variance on the SAS program on an IBM 4381 computer.

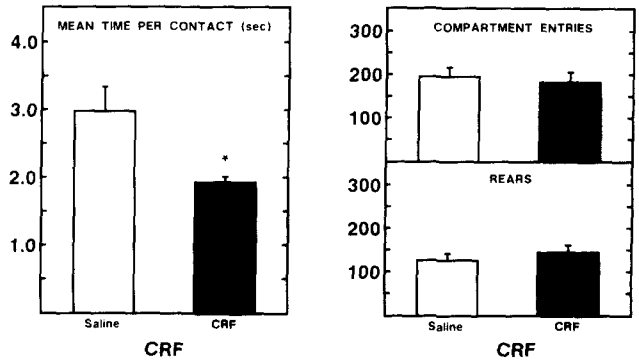

FIG. 1. The effect of ICV CRF on behavior in rats. Animals were injected into the lateral ventricle with 25 ng of CRF (N=8) or saline (N=9) 10 minutes before testing in the multicompartment chamber. The number of rears, compartment entries and the mean duration of the contact with the stimulus (a wire-mesh sphere) are shown (scorer HAB). The CRF-treated animals showed a significant decrease in the mean time per contact ($p<0.05$, Dunnett's t-test). There were no statistically significant differences in compartment entries or rears.

RESULTS

Because our previous data had been obtained from mice, we first tested the effects of lateral ventricle administration of 25 ng CRF on the behavior of rats in the MCC. Figure 1 shows that the mean duration of contacts with the wire-mesh stimuli of rats receiving CRF was significantly decreased compared to saline-injected controls. At doses of 50 ng or higher locomotor activity was markedly reduced. None of the other behavioral measures (compartment entries, rears, and grooming) were significantly altered. Thus, these results resemble those observed previously in mice following ICV administration of CRF (3).

We then tested the effect of cold cream blockade of the cerebral aqueduct on the response to CRF. Cold cream was injected either in the third ventricle immediately rostral to the aqueduct or into the fourth ventricle immediately caudal to the aqueduct. CRF was injected either into the lateral ventricles, or the fourth ventricle. Examination of the sagittal sections postmortem indicated whether or not the cerebral aqueduct was blocked by the cold cream, assisted by the presence of the dye marker. We thus derived four groups of animals: those in which the cerebral aqueduct was blocked with CRF injected into the lateral or fourth ventricles, and those in which the aqueduct was not blocked with administration of CRF in the lateral or fourth ventricles. Figure 2 shows the data on mean stimulus-contact times of 42 rats. The behavioral scores, including the mean-contact times, are very similar to those observed in Fig. 1, suggesting that there were no toxic effects of the cold cream injections. In fact, stimulus-contact times of rats injected with saline and not Nivea (3.26±0.19) were not significantly different from those that were injected with Nivea (3.01±0.24). Moreover, Nivea-injected rats also injected with saline that had complete blocks of the aqueduct did not display stimulus-contact times (3.22±0.28) significantly different from those that had incomplete blocks (2.94±0.21), suggesting that if any hydrocephalus occurred, it did not alter the behavioral scores. Furthermore, CRF still decreased stimulus-contact times, and was equally effective in decreasing stimulus-contact times when injected either into the lateral or the fourth ventricles. Lateral

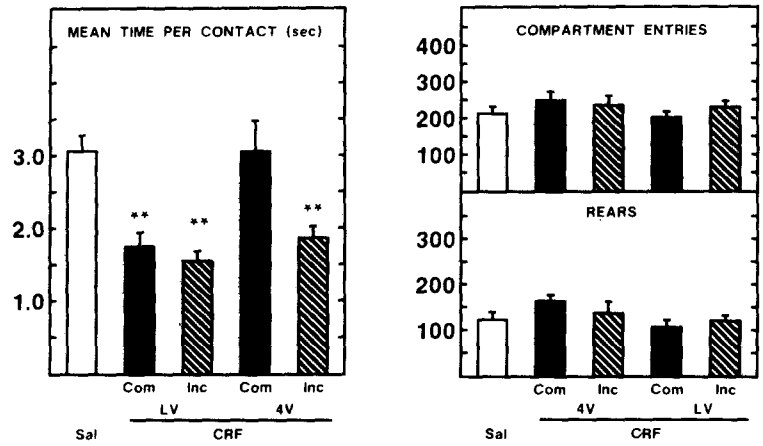

FIG. 2. The effect of cold cream injections in the area of the cerebral aqueduct on CRF-induced behavior in the MCC. Animals were divided into those that showed complete (Com) blocks of the aqueduct, and those that did not (Inc). CRF (20 ng) or saline was injected into the lateral (LV) or fourth ventricles (4V). The saline (Sal) group includes animals that received saline in either the lateral or the fourth ventricles, and had either complete or incomplete blocks of the cerebral aqueduct, among which no statistically significant effects were found. Behavior was scored by CWB. $^{**}p<0.01$ compared to saline controls (Dunnett's t-test).

ventricle injections of CRF were effective whether or not the cerebral aqueduct was blocked. However, fourth ventricle injections of CRF were not effective when the cerebral aqueduct was blocked. ANOVA indicated a significant main effect of the site of CRF injection, $F(1,29)=13.7$, $p<0.001$, of whether or not the blockade of the cerebral aqueduct was effective, $F(1,29)=8.2$, $p<0.01$, and a significant interaction between these two factors, $F(1,29)=4.2$, $p<0.05$. This significant interaction suggests that the cold cream block alters the behavioral response to CRF. Post hoc analyses indicated that all groups except the fourth ventricle-complete block were significantly different from saline (Dunnett's t-test). Duncan's and Scheffé's tests indicated that there was a statistically significant difference between the incomplete and complete blocks with fourth ventricle CRF injections. No significant differences were observed among the various groups in the other behavioral measures. We conclude that the site of this action of CRF is not in the fourth ventricle, and appears to be in the third or lateral ventricles.

We then studied the effects of cold cream injected into various parts of the third ventricle on lateral ventricle injection of CRF. A total of 38 rats was studied in this way. To facilitate the analysis of the data, the third ventricle was arbitrarily divided into four contiguous areas, as depicted in Fig. 3. Area 1 included the dorsal horizontal tract of the third ventricle and extended rostrally to the subfornical organ (SFO). Area 2 included the rostral vertical part of the third ventricle. Area 3 was the anteroventral portion of the

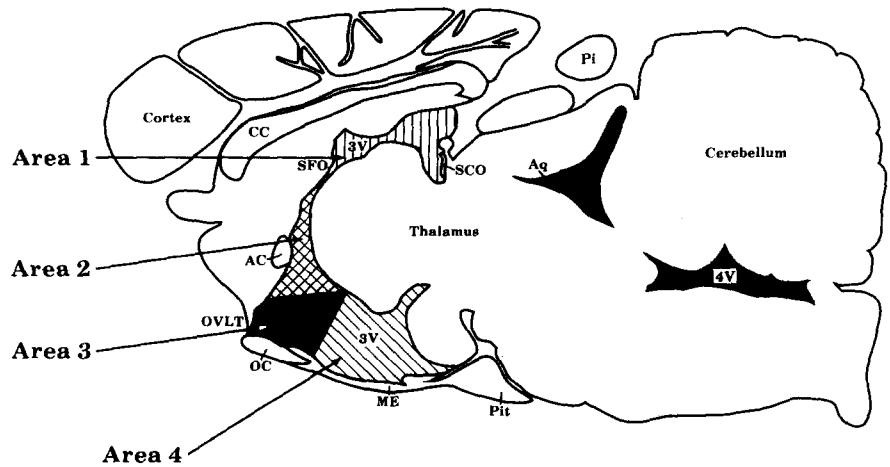

FIG. 3. Diagram depicting the extent of the third ventricle Areas 1, 2, 3, and 4 used to analyze the data. 3V, third ventricle; 4V, fourth ventricle; AC, anterior commissure; Aq, cerebral aqueduct; CC, corpus callosum; ME, median eminence; OC, optic chiasm; OVLT, organum vasculosum laminae terminalis; Pi, pineal gland; Pit, pituitary; SCO, subcommissural organ; SFO, subfornical organ.

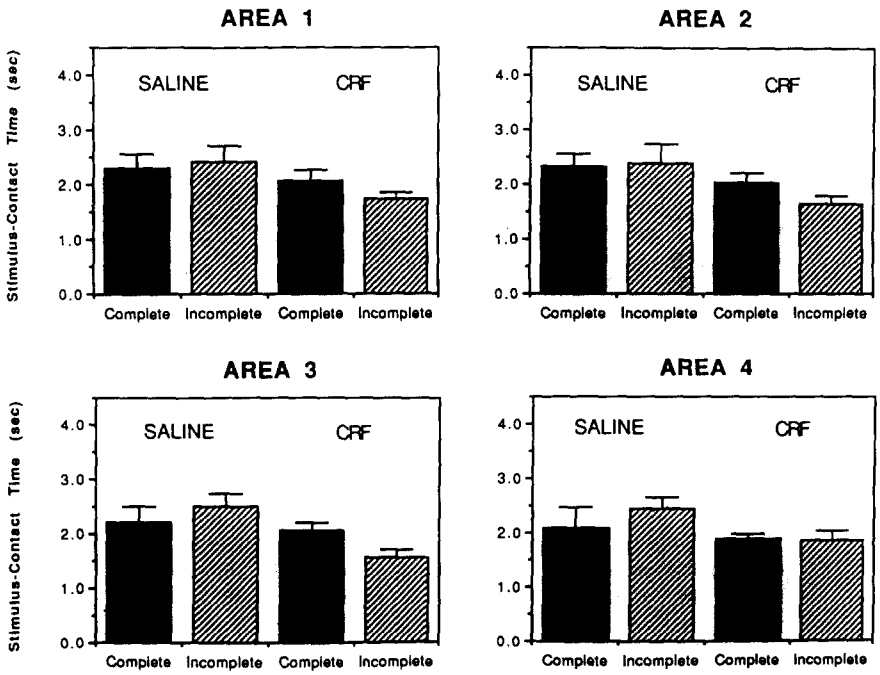

FIG. 4. The effect of blockade of different areas of the third ventricle on CRF-induced behavior in the MCC. Behavior was scored by FS. Analysis of variance indicated a significant effect of CRF on stimulus-contact times in Areas 1, 2, and 3, but not 4. There was a statistically significant interaction between the cold cream block and CRF only in Area 3, suggesting that this was the only region in which a cold cream block attenuated the behavioral response to CRF. Area 3 was also the only region in which there was a statistically significant difference between complete and incomplete blocks in CRF-injected rats. There were no statistically significant differences in compartment entries or rears.

third ventricle (AV3V), essentially the area surrounding the organum vasculosum laminae terminalis (OVLT). Area 4 was the ventral horizontal tract of the ventricle dorsal of the median eminence. Area 1 included the subcommissural organ (SCO) and the SFO, Area 3 the OVLT and the most rostral portion of the median eminence, and Area 4 the median eminence.

The data were analyzed in terms of the four areas of the third ventricle blocked by the cold cream (Fig. 4). The mean stimulus-contact times in this experiment were lower than those obtained in the earlier ones, probably because this study was conducted in a different laboratory (Shreveport rather than Gainesville), on rats from a different supplier (Harlan rather than University of Florida) and with a different scorer. In pilot studies, stimulus-contact times from saline-injected rats (1.85 ± 0.23) did not differ from saline-injected rats that had also been injected with Nivea (2.36 ± 0.21). However, as in the earlier studies, ICV CRF significantly decreased mean stimulus-contact times. Because the cold cream injections were fairly large, most rats had more than one area blocked, so that the behavioral data from one animal are in most cases included in more than one group. It is evident from Fig. 4 that injections of cold cream in Area 4 had no effect on the CRF-induced changes. Moreover, injections in Areas 1, 2 and/or 3 attenuated the ability of CRF to decrease mean contact times. The results of the statistical analysis are presented in Table 1. ANOVA indicated statistically significant effects on stimulus-contact times for CRF in animals with cold cream blocks in third ventricle Areas 1, 2, and 3, but not Area 4. If blockade of a particular area prevented the effect of CRF, then there should be a significant interaction between the cold cream block and the CRF injection. A statistically significant interaction was found only for Area 3, the interaction was not statistically significant for Areas 1, 2 or 4. A further indication of the selectivity of the effect for Area 3 is provided by post hoc *t*-tests which indicated statistical significance for the difference between complete and incomplete blocks in CRF-treated rats, only in Area 3, $t(23) = 2.4$, $2p < 0.05$. Results for the other areas were not statistically significant [Area 1: $t(23) = 1.4$; Area 2: $t(23) = 1.7$; Area 4: $t(23) = 0.05$, all $2p > 0.05$]. This suggests that Area 3 is the crucial area for the behavioral actions of CRF in the MCC.

DISCUSSION

Our results indicate that ICV CRF is able to induce a stress-like response on exploratory behavior in the rat, just as we had observed previously in mice (3). The results of the cerebral aqueduct blocks with cold cream were clear. Injections of CRF

TABLE 1

STATISTICAL ANALYSIS OF THE EFFECTS OF THIRD VENTRICLE COLD CREAM BLOCKS ON CRF-INDUCED EXPLORATORY BEHAVIOR

Area Blocked	Effect of CRF	CRF/Area Interaction
Area 1	$F(1,34) = 4.6$, $p < 0.05$	$F(1,34) = 1.2$, $p > 0.1$
Area 2	$F(1,34) = 5.1$, $p < 0.05$	$F(1,34) = 1.0$, $p > 0.1$
Area 3	$F(1,34) = 7.2$, $p < 0.01$	$F(1,34) = 3.8$, $p < 0.05$
Area 4	$F(1,34) = 3.2$, $p > 0.1$	$F(1,34) = 0.7$, $p > 0.1$

Two-way ANOVA was performed on the data of Fig. 4 to determine the effects of CRF and blockade by cold cream on each of the third ventricle areas defined in Fig. 3.

into the lateral ventricles were behaviorally active whether or not the cerebral aqueduct was blocked, whereas fourth ventricle injections were effective only when the cerebral aqueduct was not blocked. These results strongly suggest that the site of action of CRF to alter exploratory behavior in the MCC is not in the fourth ventricle.

The data obtained with third ventricle injections of cold cream suggest that the site of action of CRF is in the third ventricle, and that it is not in the ventrocaudal aspect. Although the data are not absolutely definitive, the statistical analysis clear implicates the AV3V (the area we designated Area 3) as the area of the third ventricular surface most clearly involved. This area includes the OVLT, a structure which has been implicated in the actions of other peptides because it does not possess a blood-brain barrier. It is possible that the effect of the cold cream was to interfere with the action of CRF in a nonspecific manner, unrelated to its physical blockade of the ventricular surface. However, this was not evident in the behavioral results, and cold cream injected into the third ventricle did not antagonize the effects of CRF in all cases.

Interestingly, blockade of the cerebral aqueduct antagonized the increased locomotor activity observed when CRF was injected into the cisterna magna, but not the lateral ventricles (20). Thus, at this low level of anatomical resolution, the effect of CRF (1 μg) on locomotor activity may share a common site with that on exploratory behavior.

It is also possible that the OVLT does not represent the site of action of CRF, but it is a region in which the neurohormone is taken up and transported to other areas. However, the site responsible for the effect is unlikely to be far from the OVLT because of the rapid onset of the behavioral change (around 5–10 minutes) after ICV CRF injection. The OVLT is a region well connected to a number of different brain nuclei including the median preoptic nucleus, the medial preoptic and anterior hypothalamic areas, and the SFO (6,14).

The AV3V has been recognized as the site of action of many neuropeptides: angiotensin II to induce drinking (12), bradykinin to alter blood pressure (13), and ACTH to induce grooming (9). The present data add CRF to this list of peptides acting on the AV3V, and suggest that it may be a site through which neuropeptides present in cerebrospinal fluid can alter a variety of brain functions.

REFERENCES

1. Arnsten, A. F. T.; Segal, D. S. Naloxone alters locomotion and interaction with environmental stimuli. Life Sci. 25:1035–1042; 1979.
2. Arnsten, A. F. T.; Berridge, C. W.; Segal, D. S. Stress produces opioid-like effects on investigatory behavior. Pharmacol. Biochem. Behav. 22:803–809; 1985.
3. Berridge, C. W.; Dunn, A. J. Corticotropin-releasing factor elicits naloxone-sensitive stress-like alterations in exploratory behavior in mice. Regul. Pept. 16:83–93; 1986.
4. Berridge, C. W.; Dunn, A. J. A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice. Horm. Behav. 21:393–401; 1987.
5. Brown, M. R.; Fisher, L. A. Corticotropin-releasing factor: effects on the autonomic nervous system and visceral systems. Fed. Proc. 44:243–248; 1985.
6. Camacho, A.; Phillips, M. I. Horseradish peroxidase study in rat of the neural connections of the organum vasculosum of the lamina terminalis. Neurosci. Lett. 25:201–204; 1981.
7. Dunn, A. J.; Berridge, C. W. Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholaminergic systems. Pharmacol. Biochem. Behav. 27:685–691; 1987.
8. Dunn, A. J.; Berridge, C. W. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res. Rev., in press; 1990.
9. Dunn, A. J.; Hurd, R. W. ACTH acts via anterior third ventricle site to elicit grooming behavior. Peptides 7:651–657; 1986.
10. Dunn, A. J.; Berridge, C. W.; Lai, Y. I.; Yachabach, T. L. CRF-induced excessive grooming behavior in rats and mice. Peptides 8:841–844; 1987.
11. Herz, A.; Alburst, K.; Matys, J.; Schubart, P.; Taschenachi, H. S. On the central sites for the antinociceptive action of morphine and fentanyl. Neuropharmacology 9:539–551; 1970.
12. Hoffman, W. E.; Phillips, M. I. Regional study of cerebral ventricle sensitive sites to angiotensin II. Brain Res. 110:313–330; 1976.
13. Lewis, R. E.; Phillips, M. I. Localization of the central pressor action of bradykinin to the cerebral third ventricle. Am. J. Physiol. 247: R63–R68; 1984.
14. Lind, R. W.; VanHoesen, G. W.; Johnson, A. K. An HRP study of the connections of the subfornical organ of the rat. J. Comp. Neurol. 210:265–277; 1982.
15. Matsuzaki, I.; Takamatsu, Y.; Moroji, T. The effects of intracerebroventricularly injected corticotropin-releasing factor (CRF) on the central nervous system: behavioural and biochemical studies. Neuropeptides 13:147–155; 1989.
16. Morley, J. E.; Levine, A. S. Corticotropin releasing factor, grooming and ingestive behavior. Life Sci. 31:1459–1464; 1982.
17. Rivier, C.; Vale, W. Mediation by corticotropin releasing factor (CRF) of adenohypophyseal hormone secretion. Annu. Rev. Physiol. 48:475–494; 1986.
18. Sirinathsinghji, D. J. S.; Rees, L. H.; Rivier, J.; Vale, W. Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat. Nature 305:232–235; 1983.
19. Sutton, R. E.; Koob, G. F.; Le Moal, M.; Rivier, J.; Vale, W. Corticotropin releasing factor produces behavioral activation in rats. Nature 297:331–333; 1982.
20. Tazi, A.; Swerdlow, N. R.; Le Moal, M.; Rivier, J.; Vale, W.; Koob, G. F. Behavioral activation by CRF: evidence for the involvement of the ventral forebrain. Life Sci. 41:41–49; 1987.
21. Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science 213:1394–1397; 1981.
22. Valentino, R. J.; Foote, S. L.; Aston-Jones, G. Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. Brain Res. 270:363–367; 1983.